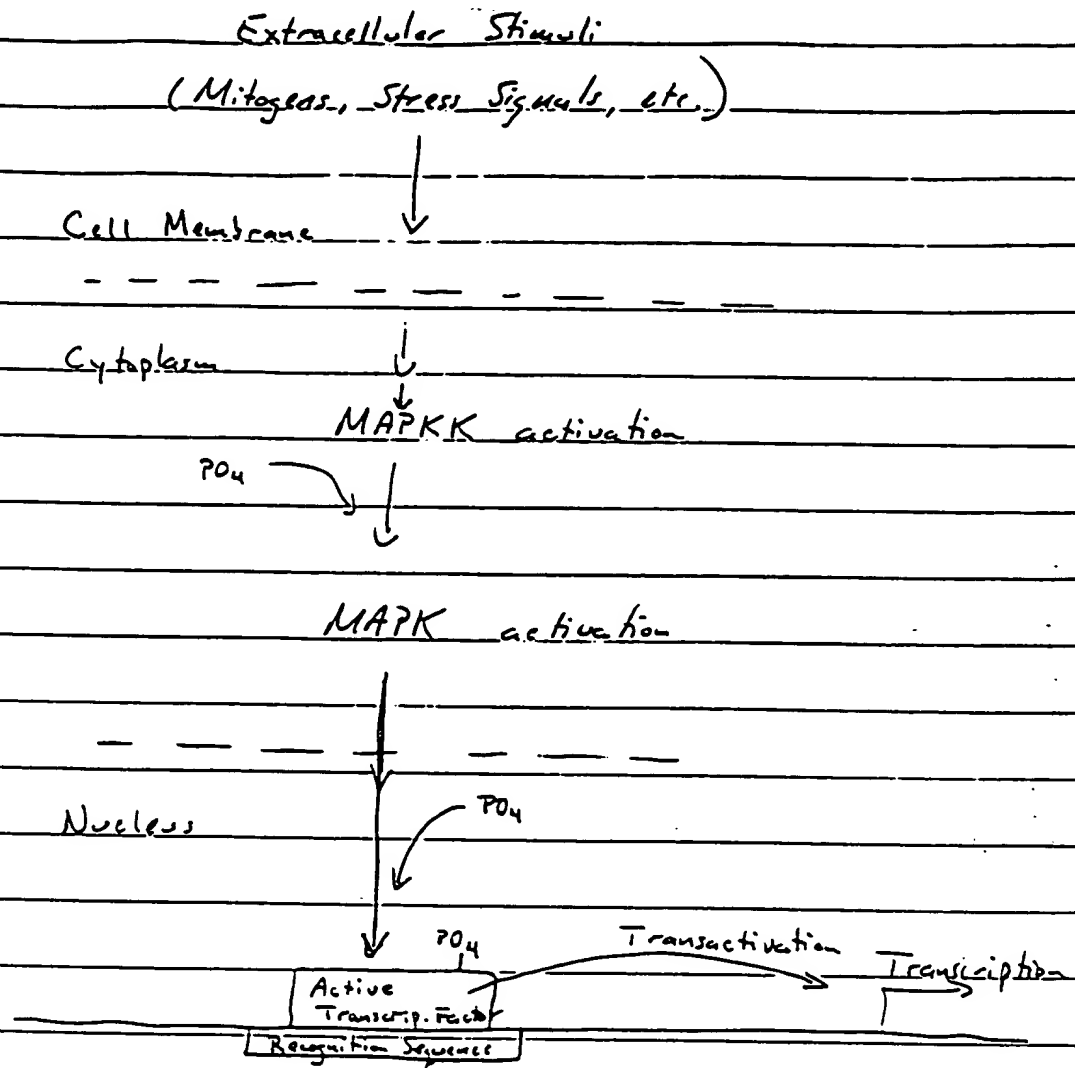


Figure



00780-05525960

Main Spring Pathway - Specific Signal Transduction

Transducer Plasmid

DBD: Pathway Specific AD -

+ Reporter Plasmid

5x DBD -

Reporter Gene -

Gene of Interest Plasmid
Promoter - Gene of Interest

Transfected Cells

Gene Product of Interest

DBD AD

activation

Reporter Gene

5x DBD
Regulatory Site

Fig 2

09637550.081.100

Common

Selection of Stable Reporter Cell Lines

Reporter Construct (w/ linked DBD element)



Stably transfect



Screen for clones with low background
of Reporter activity and strong
response to DBD-bearing activator(s)



"Stable Reporter Cell Line"



Stably transfect with fusion
transactivator plasmid



Screen for clones with strong
response to pathway-specific
upstream activator(s)



"Pathway-Specific Stable Reporter Cell Line"

00637550-081100

4.1.1. pFR-Luc Plasmid



Fig. 4

Sequence of GAL4 Binding Element in the pFR-Luc Plasmid

GT CCGAGTACTGTCCTCCG AG CCGAGTACTGTCCTCCG
 AG CCGAGTACTGTCCTCCG AG CCGAGTACTGTCCTCCG
 AG CCGAGTACTGTCCTCCG AG CCGAGACTCTAGAGGG
 TATATATGGATCCCCGGGT AC CGAGCTCGAATTC--
 --CAGCTTGGCATTCCGGTACTGTTGGTAAATG--Luciferase

4.1.2. Fusion Transactivator Plasmids

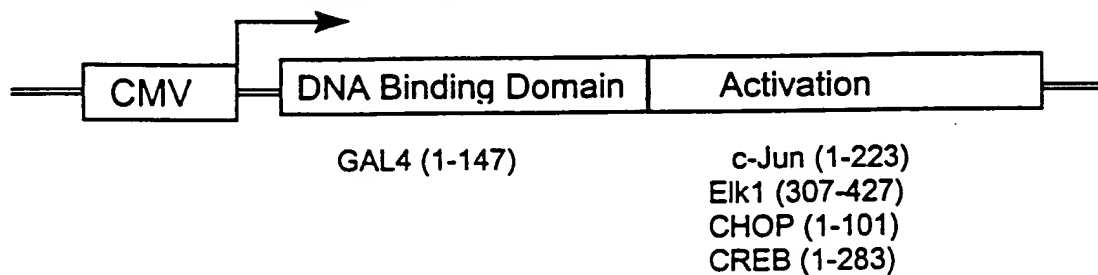


Figure 5

4.1.3. Control Plasmids

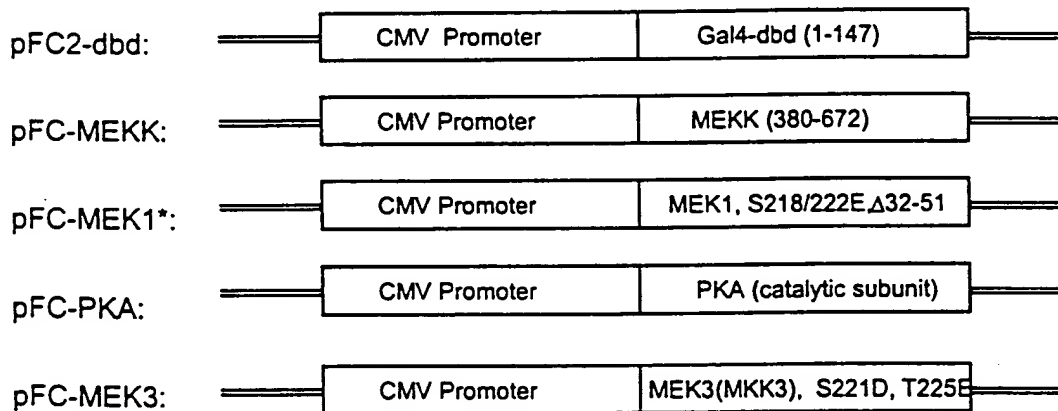


Figure 7

will tailor
to each application

4.1.4. pFA-CMV Plasmid

pFA-CMV Plasmid

*Bam*HI | *Srf*I | *Sma*I | *Eco*R | *Xba*I | *Hind*III | *Pst*I | *Sac*I | *Kpn*I | *Bgl*II
 GTA TCG CCG GGA TCC GCC CGG GCT GGA ATT CTA GAA GCT TCT GCA GAG CTC GGT ACC AGA TCT TGA ATA AGT AG
 V S P G S G R A G I L E A S A E L G T R S * * *

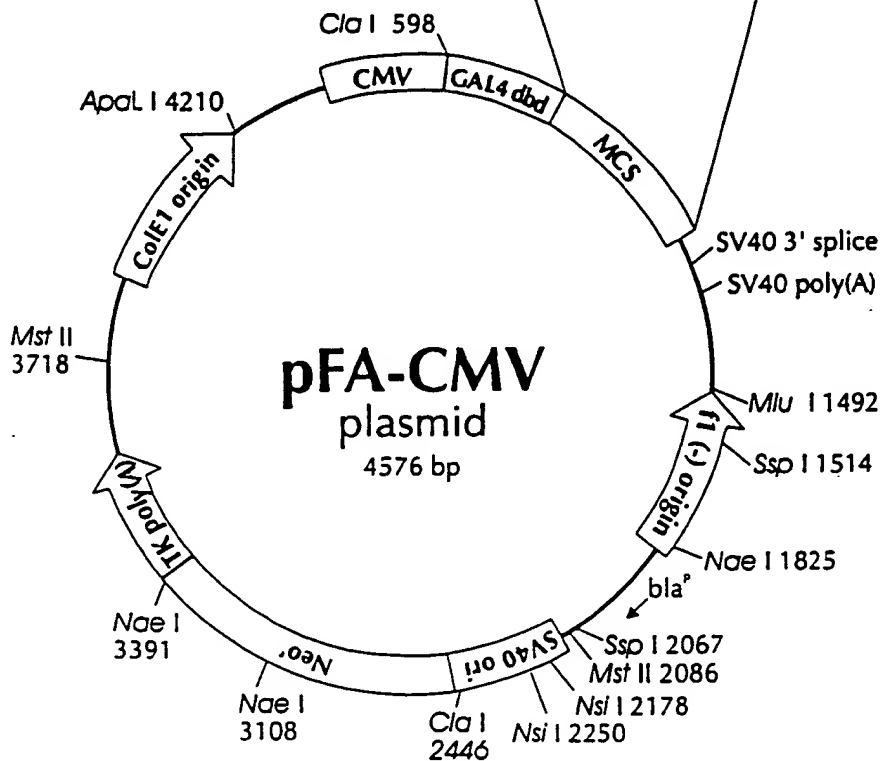


Figure 6

4.2. Preparation of medium and reagents

Luciferase Assay Reagent (1×)

40.0 mM tricine (pH 7.8)
 0.5 mM ATP
 10 mM MgSO₄
 0.5 mM EDTA
 10.0 mM DTT
 0.5 mM coenzyme A
 0.5 mM luciferin

Cell Lysis Buffer (5×)

40 mM tricine (pH 7.8)
 50 mM NaCl
 2 mM EDTA
 1 mM MgSO₄
 5 mM DTT
 1% Triton® X-100

00180"0552E950

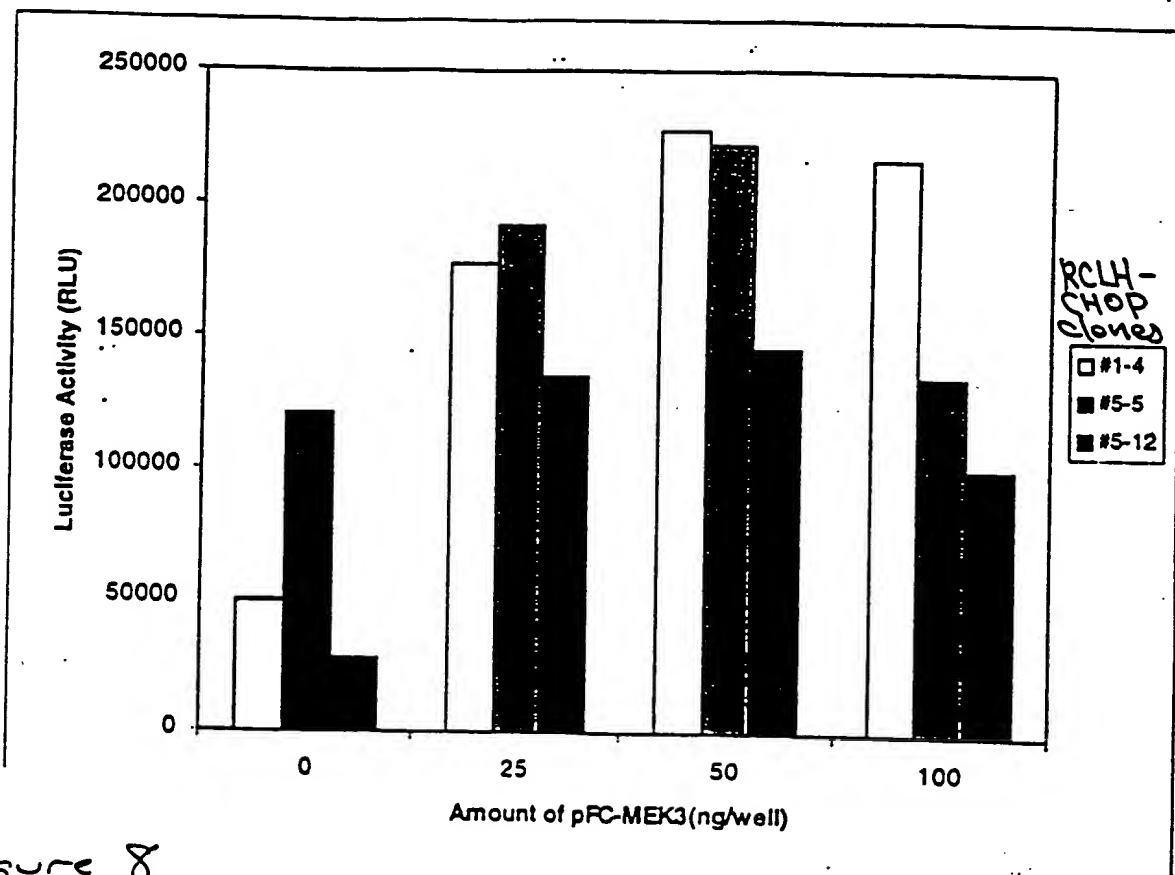


Figure 8

001130-0557950

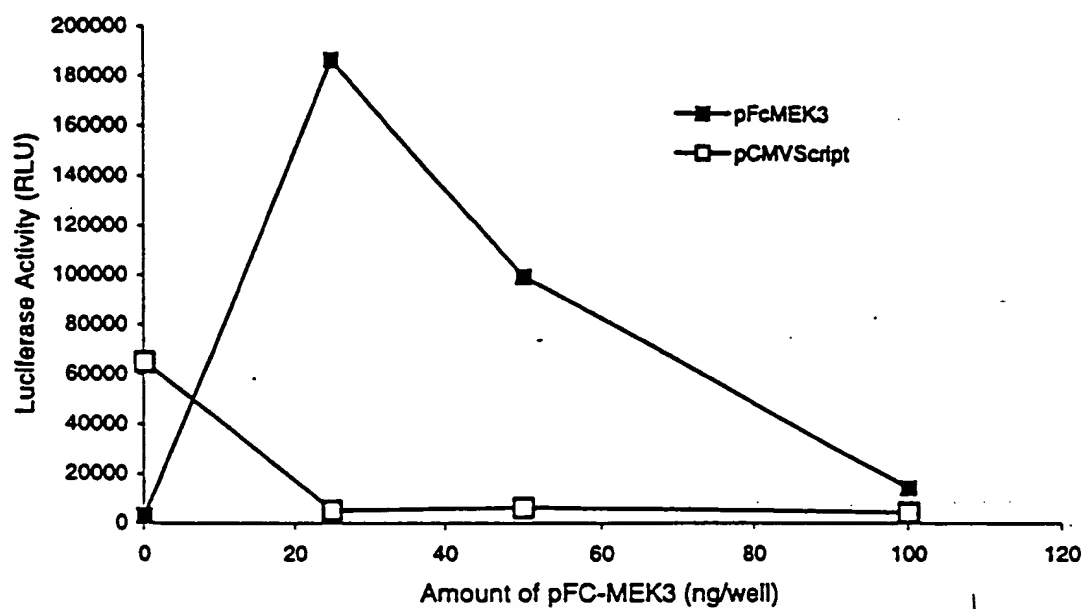


Figure 9

00780" 0552E960

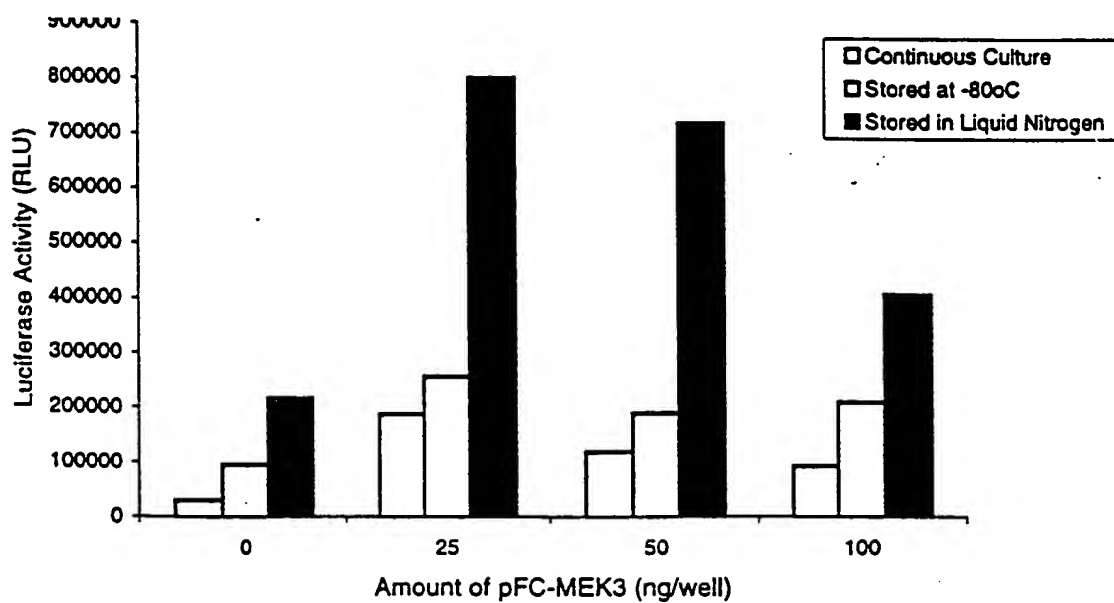


Figure 10